JOURNAL OF PURE AND APPLIED MICROBIOLOGY, APRIL 2014. Vol. **8**(2), p. 1755-1758

Multiplex PCR Detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in Skin of Poultry

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(Received: 12 February 2014; accepted: 23 March 2014)

The aim of the present study was to investigate the presence *Arcobacter*, an important emerging bacterial pathogen having public health significance, in poultry skin samples by using multiplex polymerase chain reaction (PCR) technique. A total number of 153 poultry skin samples were collected from local retail shops of Bareilly region, Uttar Pradesh, India. All the samples were inoculated into Arcobacter enrichment broth with Cefoperazone, amphotericin B and teicoplanin (CAT) supplement and incubated micro-aerobically at 30º C for 48 hrs. The DNA extraction from all the enriched samples was done by snap chill method and subjected to multiplex PCR (m-PCR) targeting both 16S rRNA and 23S rRNA genes of *Arcobacter*. Out of the 153 enriched samples, 35 samples (22.88%) were found positive by m-PCR with amplification products of 401 bp size and 257 bp which were specific for two species of Arcobacters viz., *Arcobacter butzleri* and *Arcobacter cryaerophilus,* respectively. This is the first report regarding identification and occurrence of Arcobacters in skin samples of poultry from India. It is suggested to consume poultry meat after removing skin so as to avoid enteric and food-borne pathogens like Arcobacters. Further detailed epidemiological studies are suggested to know the prevalence and magnitude of *Arcobacter* species, which would help in designing and adapting suitable prevention and control measures to counter this important pathogen.

Key words: *Arcobacter*, skin, poultry, food-borne pathogen, prevalence, multiplex PCR.

Arcobacters are emerging food-borne pathogens, under *Campylobacteraceae* family, reported worldwide and have been implicated with several disease manifestations in animals like mastitis, diarrhoea, abortion and reproductive problems, and also reported to have public health concerns[1,2,3]. The two important species of Arcobacters are *Arcobacter butzleri* and *Arcobacter cryaerophilus*. *Acrobacter* spp. which have been associated with enteritis and bacteraemia in animals and humans[4,5,6,7]. Contaminated water and meat play an important role in the transmission of *Arcobacter*[1,7,8,9,10,11]. *Arcobacter* spp have been isolated from various foods of animal origin such as chicken meat, beef and pork, and water resources[2,12,13,14,15,16].

Only few reports regarding prevalence of Arcobacters from India have been documented in recent years from clinical diarrhea cases in humans[17,18] and from different animals, humans and

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food sources[2]. Arcobacters have not been reported from skins of poultry, therefore the present study was designed to detect the presence of Arcobacters in poultry skin samples collected from retail meat shops of Bareilly region of Uttar Pradesh, India, employing the molecular tool of multiplex polymerase chain reaction (m-PCR).

MATERIALS AND METHODS

Sample collection and Processing

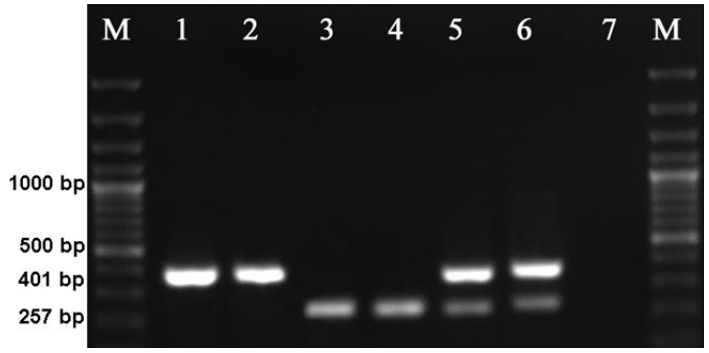
A total of 153 poultry skin samples were collected from different retail meat shops of the Bareilly region, Uttar Pradesh, India. Ten gram of these poultry skin samples were aseptically minced separately with scissors and suspended in 90 ml of phosphate buffer saline (PBS, pH 7.2). The mixtures were homogenized with stomacher for 1 min at 200 rpm, and 1 ml of suspension was inoculated into 10 ml of Cefoperazone, amphotericin B and teicoplanin (CAT) broth and incubated at 30°C under microaerophilic condition for 48 hrs.

Multiplex PCR (m-PCR) detection of *Arcobacter* species

After enrichment, whole cell DNA was extracted from all the processed skin samples by heat lysis (snap chill) method and were individually subjected to optimized protocols of multiplex specific PCR for detection of the presence of *Arcobacter* spp. (*Arcobacter butzleri* and *Arcobacter cryaerophilus*) as per Houf *et al*.[19] with slight modification of the original protocol. Primer sets used were BUTZ, ARCO, CRY-1, and CRY-2, designed from 16S rRNA and 23S rRNA genes. The 50 µl reaction mixture was composed of 5 µl of 10x PCR buffer, 2.5 U of *Taq* DNA polymerase, 0.2 mM of each deoxyribo nucleotide triphosphate, 2.5 mM $MgCl_2$, 30 pmol of each primers (ARCO, BUTZ, CRY-1 and CRY-2) 8 µl heat lyses DNA of the bacteria as template and the final volume was adjusted to 50 µl with Nuclease free water. The multiplex PCR was performed with an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation (94°C for 30 sec.), primer annealing (51°C for 30 sec) and extension (72°C for 1.00 min) and final extension at 72°C for 10 min. The PCR product was subjected to electrophoresis on 1.5% agarose gel made in TAE (Tris-acetate-EDTA) buffer and analyzed by using UV trans-illuminator (Gel-Doc system).

RESULTS AND DISCUSSION

Out of 153 poultry skin samples tested, 35 samples (22.88%) were found to be positive for *Arcobacter* species by multiplex PCR with an amplification product of 401 bp and 257 bp, specific only for *Arcobacter butzleri* and *Arcobacter cryaerophilus*, respectively. Out of 35 positive samples, 12 samples were positive for *A. butzleri*, and 17 for *A. cryaerophilus*, while 6 revealed mixed infection of these two *Arcobacter* species. Arcobacters have been frequently isolated from food products of animal origin (chickens, pork, beef and lamb). For Arcobacters, m-PCR has been employed for detection of Arcobacters at species level by several workers[1,2,3]. *Arcobacter* species have been implicated as important food-borne pathogens due to their detection in various foods of animal origin, especially in products from chicken all over the world[2,3,14,20,21,22]. The highest prevalence rate has been found in chickens (23%), followed by pork (7%) and beef (2.2%) from retail shops in Japan for *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*[14]. A higher prevalence rate of 73% in chickens, 29% in pork, 22% in beef and 15% of the lamb samples has been reported for *A. butzleri* from Australia[21]. Recently, PCR testing showed 25.33% prevalence rate of Arcobacters in pig and 17.33% in chickens, while in food samples the prevalence rates were of 21.33% in sea foods, 16% in pork and 12% in chicken meat[2]. *A. butzleri* has been found to have higher prevalence rate in meat samples as compared to *A. cryaerophilu*[23,14,15,16]. In the present study, a 22.88% prevalence rate of Arcobacters in poultry skin samples indicates varying and moderate prevalence as compared to earlier reports[2,14,21], and contrary to these earlier reports significantly out of 35 positive poultry skin samples, a higher number of 17 were positive for *A. cryaerophilus*, while a lesser number of 12 being positive for *A. butzleri*. Compared to conventional cultural isolation and detection, PCR testing of the samples for Arcobacters has been reported to be rapid, easy and less time consuming. The PCR version of multiplex PCR (m-PCR) has added advantage of detecting a specific pathogen amongst others, ability to detect and differentiate upto species or strain level of any pathogen. The present study adds to the epidemiological data available for Arcobacters and signifies the



Lane M: Molecular weight marker, 100 bp plus
Lane 1, 2: *Arcobacter butzleri* (401 bp)
Lane 3,4: *Arcobacter cryaerophilus* (257 bp)
Lane 5,6: Mixed infection of *Arcobacter butzleri* (401 bp) and *Arcobacter cryaerophilus* (257 bp)
Lane 7: Negative control

Fig. 1. Multiplex PCR detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in skin samples of poultry in agarose gel electrophoresis

occurrence of Arcobacters in poultry skin samples which may have food-borne disease implications. The study also supports the recently finding of Patyal *et al*.[2] which revealed *Arcobacter* spp. to be emerging in the country in animals and various foods (particularly meat) of animal origin.

CONCLUSION

This seems to be the first report of occurrence of *Arcobacters* in poultry skin samples with a moderate prevalence rate of 22.88%, which reveals that of *Arcobacter* species may be highly prevalent in poultry skin that may act as important source contamination for chicken meat and further a source of food-borne infection to human. Possibilities of Arcobacters cross-contamination of poultry skin during slaughtering / processing at retail shops needs to be investigated in details. Consumption of poultry meat after removing the skin is suggested to prevent food-borne pathogens like of Arcobacters. The prevalence and magnitude of *Arcobacter* species need to be studied with wide epidemiological investigations in the country and specifically its food-borne implications which would help in devising suitable prevention and control strategies to combat this significant pathogen having zoonotic and public heallth concerns.

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